wherein the nucleic acid molecule comprising a TMS1 CpG island is selected from the group consisting of

(a) nucleic acid molecules which hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4, and

(b) complements of (a), and

wherein an increase in the level of methylation of the nucleic acid molecule comprising a TMS1 CpG island in the biological sample compared to the control identifies a subject who is at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy.

Remarks

Claims 1, 47 and 110-124 are currently pending. Claims 1, 47 and 118-122 have been amended. New claims 123 and 124 have been added.

Claim 1 has been amended to replace the preamble term "abnormal methylation" with "abnormally increased methylation." Abnormally increased methylation means a level of methylation above that level observed in a control. Support for this amendment can be found throughout the specification and particularly in the body of claim 1 and on page 2, lines 21-23; and page 39, lines 30-33.

Claim 1 has been further amended to replace the term "tumor" with the term "cancer" in order to embrace the identification of subjects at risk of developing solid mass as well as non-solid mass cancers. Support for this amendment can be found throughout the specification and particularly at page 41, lines 21.

Claims 47 and 118-122 have been amended to characterize the anti-cancer therapy as an apoptosis-dependent anti-cancer therapy. An "apoptosis-dependent anti-cancer therapy" is an anti-cancer therapy that induces apoptosis of cells, or requires apoptosis in order to be effective. One example of an apoptosis-dependent anti-cancer therapy is a DNA damaging therapy. Support for these amendments can be found throughout the specification and particularly at page 9, lines 14-18; and page 55, lines 16-24.

New claims 123 and 124 are similar to claims 1 and 47, respectively. The difference between the new claims and those previously pending is that the level of methylation is determined in a nucleic acid molecule comprising a TMS1 CpG island, regardless of whether that nucleic acid molecule also encodes a native TMS1 polypeptide. The specification teaches that the TMS1 genomic locus is methylated at its CpG island, which is located 5' of the TMS1 coding sequence. The specification further provides that SEQ ID NO:4 comprises the nucleotide

sequence of the TMS1 CpG island. Accordingly, methylation analysis can be carried out with nucleic acid molecules that bind to complements of SEQ ID NO:4 (and complements thereof). Support for these new claims can be found throughout the specification and particularly in claims 1 and 47 together with the teachings on page 14, lines 27-28; page 16, lines 10-15; and page 20, lines 27-35.

Applicants previously paid for 17 independent claims (i.e., 14 additional independent claims paid for in excess of 3) and 20 total claims. There are currently 4 independent claims and 17 total claims pending. Accordingly, no additional fees are due as a result of these claim additions.

No new matter has been added.

The Claimed Invention

The invention as currently claimed relates to diagnostic and prognostic methods, based on TMS1 methylation status. One method relates to the identification of subjects at risk of developing a cancer. Another method relates to identifying subjects having cancer who are at risk of being non-responsive to an anti-cancer therapy.

The methods involve an analysis of methylation at the TMS1 genomic locus. More specifically, the level of methylation of the TMS1 genomic locus in a biological sample from a subject is determined and compared to the level of methylation in a control. An increase in the level of methylation in the subject sample as compared to the control is indicative of either a subject at risk of developing a cancer, or a subject at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy. Methylation at the TMS1 genomic locus can be measured using nucleic acid molecules that hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4, which may code for a native TMS1 polypeptide, and complements thereof.

Methylation levels can be determined using a number of different techniques, such as those taught in the specification and those known to persons of ordinary skill in the art. The control can be derived from normal tissue from a normal subject, or normal tissue from a subject having cancer. The apoptosis-dependent anti-cancer therapy can include a DNA damaging anti-cancer therapy, radiation therapy, or chemotherapy. In one embodiment, the method further comprises administering to the subject so identified a demethylating agent and an apoptosis-dependent anti-cancer therapy. In still another embodiment, the method further comprises administering to the subject so identified an anti-cancer therapy that is not dependent upon

apoptosis such as a biological response modifying therapy, immunotherapy, cancer vaccine therapy, hormone therapy, or angiogenesis inhibiting therapy.

Rejection under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 1, 47 and 110-122 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. According to the Examiner, neither the specification nor the art teaches how to make and use the invention as broadly as claimed. The Examiner sets forth the following bases for her rejection:

1. Tumor analysis:

With respect to Figure 4 of the specification which illustrates that twelve of eighteen tumors analyzed showed TMS1 methylation, the Examiner states that "it is unclear whether the other 6 tumors not illustrated are consistent with the findings of the tumors or whether methylation was seen (in) normal tissues but absent in the tumors."

Applicant respectfully traverses the Examiner's rejection for the following reasons. Figure 4 of the specification illustrates a tumor and normal tissue pairing for twelve different patient samples. Five of these samples (numbered 1, 2, 3, 4, 6) demonstrate only unmethylated TMS1 in normal tissue and both unmethylated and methylated TMS1 in tumor tissue. Three of the twelve samples demonstrate both unmethylated and methylated TMS1 in normal tissue and in tumor tissue (numbered 5, 7, 8). The remaining four samples demonstrate unmethylated TMS1 in both normal and tumor tissue (numbered 9, 10, 11, 12). The twelve tumors illustrated in Figure 4 were chosen as representative of the eighteen tumors analyzed. The six remaining samples, which are not illustrated in Figure 4, did not differ significantly from those illustrated. In total, Applicant has analyzed twenty-seven breast tumors, eleven of which show TMS1 methylation. (See Appendix B, reference previously cited to Examiner.) Accordingly, TMS1 methylation is correlated with cancer development in a significant number of cancer samples.

2. Cell line analysis:

The Examiner states that "increased (sic) of SEQ ID NO:4 in cancer cell lines is not sufficient evidence to enable one skilled in the art to determine that this would necessarily be hypermethylated in primary tumor tissue as compared to non-tumor tissue." The Examiner

further relies on the Dermer et al. reference (*Biotechnology*, Vol. 12, March 1994, p. 320) to support her assertion "the level of predictability between the activity of tumor cell lines and active tumor tissue is very low" (and) "thus, the studies with cell lines, while interesting provide little insight into tumors without undue experimentation."

Applicant respectfully traverses the Examiner's rejection for the following reasons. The specification provides TMS1 methylation data from normal tissue samples and tumor samples harvested from human subjects. In some instances, the specification provides a comparison of TMS1 methylation in tumor tissue and its corresponding normal tissue counterpart. For example, Figure 4 of the specification illustrates that five out of twelve primary tumor samples contain methylated TMS1, while their normal tissue counterparts contain only unmethylated TMS1. Moreover, the reference in Appendix B documents TMS1 methylation in eleven of twenty-seven primary breast tumors analyzed, some of which are included in Figure 4. This demonstrates a correlation of TMS1 methylation and tumor presence in a significant number of subjects.

The specification also provides TMS1 methylation data from normal and tumor cell lines. Cell lines are not generally equivalent to freshly harvested tumor tissue, however, they are one of the closest in vitro approximations to freshly harvested tumor tissue. One of the caveats of cell line analysis is that the identified phenomenon is an event that occurs post-harvest of a primary sample, and may be correlative with the derivation, rather than the source, of the cell line. Cell lines can be established via a number of different molecular mechanisms, most of which involve at least one genetic mutation leading to immortalization. Immortalizing mutations are varied and random, and one would not expect to observe the same mutation in a significant proportion of cell lines. Accordingly, identification of a common molecular phenomenon in several cell lines, particularly those that are independently established, strongly suggests that the phenomenon is correlative with the cell line source (i.e., a cancer or tumor sample) rather than a post-harvest event. Applicant's analysis of *several* cell lines for each tumor type indicates that roughly 40% of tumor cell lines possess TMS1 methylation, regardless of tumor type. This strongly suggests that TMS1 methylation correlates with the risk of cancer development, a finding that mirrors the observations in primary tumor samples.

3. Cancer types:

The Examiner states that "the specification has only provided results directed to breast tumors (Figure 4)" (and that) "the identification of a CpG island indicative of breast tumors does

not provide guidance to any cancer." The Examiner further states that "not all cancers involve tumors, such as leukemia." As a result, the Examiner concludes that the skilled artisan would be required to perform additional experiments which would be undue, and the results of such experiments would be unpredictable.

Applicant has amended claim 1 to replace the term "tumor" with "cancer," and thus to embrace the diagnosis of solid tumor as well as non-solid tumor cancers. Support for this amendment can be found on page 41, lines 1-21.

Applicant respectfully traverses the Examiner's rejection in view of this amendment and the reasons set forth below. The specification demonstrated the presence of TMS1 methylation in breast cancer cells. Submitted herewith is a Declaration under 37 C.F.R. § 1.132 of the Applicant, Dr. Paula M. Vertino, with supporting data documenting TMS1 methylation patterns in normal brain tissue, glioblastoma multiforme primary tumors, lung cancer cell lines, and ovarian cancer cell lines. Approximately half (i.e., 8/17) of the glioblastoma multiforme primary tumors and none of the normal brain tissue samples demonstrated TMS1 methylation. TMS1 methylation was observed in ten of fourteen lung cancer cell lines and in sixteen of thirty-one ovarian cancer cell lines analyzed. As stated above, the analysis of these cell lines is consistent with the primary tumor data. Accordingly, Applicants have demonstrated TMS1 methylation in breast, brain, lung and ovarian cancer samples, and this is supportive of the prognostic significance of TMS1 methylation in cancer generally.

4. Abnormal methylation:

The Examiner states that "the specification does not support hypomethylation of TMS1 as indicative of risk for tumor" (and) "the claim broadly reads abnormal methylation which includes both hypermethylation and hypomethylation."

Applicant has amended claim 1 to replace the term "abnormal methylation" with "abnormally increased methylation." Abnormally increased methylation means a level of methylation above that level observed in a control. Support for this amendment can be found in the body of claim 1 and on page 39, lines 30-33 of the specification.

5. Prognostic methods:

The Examiner states that "the specification provides no guidance to determining non-responsiveness to anti-cancer therapy by detecting the hypermethylation" (and) "one means for supporting this claim would have been a study which found hypermethylation in patients at SEQ

ID NO:4 and compare the data to data with respect to patient's non-responsiveness to anti-cancer therapy." The Examiner further states that "as provided in claim 122, it is unclear why a subject at risk of being non-responsive to an anti-cancer therapy would be administered such a therapy."

Applicant has amended claims 47 and 118-122 to clarify that the anti-cancer therapy is an "apoptosis-dependent" anti-cancer therapy. To re-iterate, an apoptosis-dependent anti-cancer therapy is an anti-cancer therapy that induces apoptosis of cells, or requires apoptosis in order to be effective. One example of an apoptosis-dependent anti-cancer therapy is a DNA damaging anti-cancer therapy. Support for this amendment can be found on page 55, lines 16-24. Applicant respectfully traverses the rejection in view of this amendment and the reasons set forth below.

Claim 47 is premised, in part, on the observation that patients treated with some forms of chemotherapy or radiation therapy are, or become, unresponsive (i.e., refractory) to such therapy. Many of these therapies are dependent upon apoptosis induction in cells following, for example, DNA damage. The ability to identify subjects likely to be non-responsive to particular forms of anti-cancer therapy would be useful since it would prevent the unnecessary administration of a particular anti-cancer therapy that would be essentially ineffective. Instead, the subject could be treated with a therapy that is generally independent of apoptosis such as biological response modifying therapies, hormonal therapies, immunomodulating therapies (e.g., immunotherapeutic agents and cancer vaccines), angiogenesis inhibitors, metalloproteinase inhibitors and the like, as in claim 122. Alternatively, if the cancer is associated with TMS1 methylation, and resultant TMS1 silencing, then a demethylating agent could be co-administered with an apoptosis-dependent anti-cancer therapy in order to potentiate the latter therapy. Accordingly, the invention provides a method for identifying and discriminating between subjects more likely to benefit from apoptosis-dependent anti-cancer therapy and those less likely to benefit.

Claim 47 is further premised, in part, on the observation that TMS1 is a proapoptotic factor. A proapoptotic factor is a factor that promotes or facilitates apoptosis (i.e., programmed cell death that can be triggered by, for example, DNA damage). There are several lines of evidence that support the role of TMS1 in the promotion of apoptosis. First, TMS1 contains a carboxy-terminal caspase recruitment domain (CARD), which is commonly shared among apoptotic inducing factors. Second, transient transfection of wild-type TMS1 into cells results in apoptosis of such cells, while transient transfection of a mutant TMS1 construct with the CARD deleted does not induce apoptosis above background, i.e., vector alone. (See page 74, lines 14-24). Third, the ability of TMS1 to induce apoptosis is dependent upon caspase-9, another

proapoptotic factor. The dependence of TMS1 function on caspase-9 was suggested by the finding that DNA fragmentation and apoptosis could be blocked in a TMS1-expressing cell population in the presence of the caspase inhibitor Z-VAD-fmk. Moreover, a caspase 9 dominant negative mutant significantly inhibited TMS1-induced apoptosis, while a caspase-8 dominant negative mutant did not. This latter point is significant because caspase-9 is known to be involved in the activation of apoptosis following release of cytochrome C from the mitochondria, an almost universal phenomenon during apoptosis triggered by numerous stimuli, including DNA damage by chemotherapeutic agents.

Accordingly, the data presented in the specification support a role for TMS1 in a signaling cascade for initiating activation of caspase-9 in response to certain external stimuli. Loss of TMS1 expression, which results from TMS1 methylation, can therefore disrupt normal apoptotic responses to DNA damage or cellular stress, thus providing resistance to apoptosis-dependent anti-cancer therapies including some forms of chemotherapeutic agents.

Finally, claim 47 recites steps of determining a level of TMS1 methylation in a biological sample from a subject having cancer, and comparing this level of TMS1 methylation to that present in a control. An increase in the level of TMS1 methylation from the biological sample relative to the control identifies a subject at risk of being non-responsive to the apoptosis-dependent anti-cancer therapy. The steps of claim 47 are almost identical to the steps of claim 1. Claim 47 is enabled in view of the teaching in the specification of the characteristics of TMS1 nucleic acid molecules, the methods of measuring methylation levels, and the identification of apoptosis-dependent anti-cancer therapies. It is within the realm of the ordinary artisan to determine a level of TMS1 methylation and to compare such level of methylation to a control in order to determine whether a subject should be administered an apoptosis-dependent anti-cancer therapy or an alternative therapy. For the same reasons, and additionally based on the teaching of the nucleotide sequence of the TMS1 CpG island, new claim 124 is similarly enabled.

The Examiner suggests that the enablement requirement could be satisfied via a study of a correlation between hypermethylation in human patients and non-responsiveness to anti-cancer therapy. However, it has never been a Patent Office standard to require human trials in support of enablement.

Respectfully, the claimed methods focus on evaluating a risk of an event occurring, such as the development of a cancer or a refractory response to a therapy. The data provided by Applicant first demonstrate a correlation between TMS1 methylation and cancer development, and second support a correlation between TMS1 methylation (with a concomitant decrease in

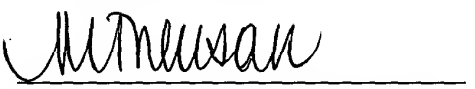
TMS1 gene expression) and an altered apoptotic responsiveness. There need not be a one-to-one correlation between TMS1 methylation and either cancer development or refractory response in order for the methods to be useful because the methods are based on assessing the risk of occurrence of these latter events. Accordingly, Applicant has met her burden of proof in establishing that TMS1 methylation status can be used to evaluate risk in a patient population.

In view of the foregoing, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection of claims 1, 47, and 110-122 under 35 U.S.C. § 112, first paragraph. For the same reasons stated above, new claims 123 and 124 are similarly enabled.

Summary

Applicant believes that each of the pending claims is now in condition for allowance. Applicant respectfully requests that the Examiner telephone the undersigned in the event that the claims are not found to be in condition for allowance. If the Examiner has any questions and believes that a telephone conference with Applicant's representative would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 720-3500 (Ext. 266).

Respectfully submitted,



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APPENDIX A:

MARKED-UP CLAIMS

1. (Amended) A method for identifying a subject at risk of developing a [tumor] cancer characterized by [abnormal] abnormally increased methylation of a CpG island containing TMS1 nucleic acid molecule comprising

determining a level of methylation of a CpG island containing TMS1 nucleic acid molecule in a biological sample from a subject, and

comparing the level of methylation of the CpG island containing TMS1 nucleic acid molecule in the biological sample to a control

wherein the CpG island containing TMS1 nucleic acid molecule is selected from the group consisting of

(a) nucleic acid molecules which hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4 and which code for a native TMS1 polypeptide, and

(b) complements of (a), and

wherein an increase in the level of methylation of the CpG island containing TMS1 nucleic acid molecule in the biological sample compared to the control identifies a subject at risk of developing the [tumor] cancer.

47. (Amended) A method for identifying a subject having cancer who is at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy comprising:

determining a level of methylation of a CpG island containing TMS1 nucleic acid molecule in a biological sample from a subject having cancer, and

comparing the level of methylation of the CpG island containing TMS1 nucleic acid molecule in the biological sample to a control,

wherein the CpG island containing TMS1 nucleic acid molecule is selected from the group consisting of

(a) nucleic acid molecules which hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4 and which code for a native TMS1 polypeptide, and

(b) complements of (a), and

wherein an increase in the level of methylation of the CpG island containing TMS1 nucleic acid molecule in the biological sample compared to the control identifies a subject who is at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy.

110. The method of claim 1, wherein the level of methylation is determined using a technique selected from the group consisting of methylation sensitive restriction analysis, methylation specific polymerase chain reaction (MSP), sequencing of bisulfite modified DNA, methylation-sensitive single nucleotide primer extension (Ms-SNuPE), and combined bisulfite restriction analysis (COBRA).

111. The method of claim 1, wherein the biological sample is breast tissue.

112. The method of claim 1, wherein the control comprises a normal tissue from a normal subject.

113. The method of claim 47, wherein the level of methylation is determined using a technique selected from the group consisting of methylation sensitive restriction analysis, methylation specific polymerase chain reaction (MSP), sequencing of bisulfite modified DNA, methylation-sensitive single nucleotide primer extension (Ms-SNuPE), and combined bisulfite restriction analysis (COBRA).

114. The method of claim 47, wherein the cancer is breast cancer.

115. The method of claim 113, wherein the biological sample is a breast cancer tumor.

116. The method of claim 47, wherein the control is normal tissue from a normal subject.

117. The method of claim 116, wherein the control is normal tissue from the subject having cancer.

118. (Amended) The method of claim 47, wherein the apoptosis-dependent anti-cancer therapy is a DNA damaging anti-cancer therapy.

119. (Amended) The method of claim 47, wherein the apoptosis-dependent anti-cancer therapy is radiation therapy.

120. (Amended) The method of claim 47, wherein the apoptosis-dependent anti-cancer therapy is chemotherapy.

121. (Amended) The method of claim 47, further comprising administering to the subject at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy, a demethylating agent and an apoptosis-dependent anti-cancer therapy.

122. (Amended) The method of claim 47, further comprising administering to the subject at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy, an anti-cancer therapy selected from the group consisting of biological response modifying therapy, immunotherapy, cancer vaccine therapy, hormone therapy and angiogenesis inhibiting therapy.

123. (New) A method for identifying a subject at risk of developing a cancer characterized by abnormally increased methylation of a nucleic acid molecule comprising a TMS1 CpG island comprising

determining a level of methylation of a nucleic acid molecule comprising a TMS1 CpG island in a biological sample from a subject, and

comparing the level of methylation of the nucleic acid molecule comprising a TMS1 CpG island in the biological sample to a control

wherein the nucleic acid molecule comprising a TMS1 CpG island is selected from the group consisting of

(a) nucleic acid molecules which hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4, and

(b) complements of (a), and

wherein an increase in the level of methylation of the nucleic acid molecule comprising a TMS1 CpG island in the biological sample compared to the control identifies a subject at risk of developing the cancer.

124. (New) A method for identifying a subject having cancer who is at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy comprising:

determining a level of methylation of a nucleic acid molecule comprising a TMS1 CpG island in a biological sample from a subject having cancer, and

comparing the level of methylation of the nucleic acid molecule comprising a TMS1 CpG island in the biological sample to a control,

wherein the nucleic acid molecule comprising a TMS1 CpG island is selected from the group consisting of

(a) nucleic acid molecules which hybridize under stringent conditions to a complement of a molecule consisting of SEQ ID NO:4, and

(b) complements of (a), and

wherein an increase in the level of methylation of the nucleic acid molecule comprising a TMS1 CpG island in the biological sample compared to the control identifies a subject who is at risk of being non-responsive to an apoptosis-dependent anti-cancer therapy.